Protective effect of *Gentiana lutea* root and leaf extracts against heterocyclic aromatic amines IQ and PhIP produced in thermally processed meat

IOP Conf. Series: Earth and Environmental Science **333** (2019) 012052 doi:10.1088/1755-1315/333/1/012052

Protective effect of *Gentiana lutea* root and leaf extracts against heterocyclic aromatic amines IQ and PhIP produced in thermally processed meat

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Abstract. During high-temperature cooking of protein rich foods, especially meat and fish, heterocyclic aromatic amines can be formed. These amines are a class of potent mutagens that can cause alterations in the structure of DNA and chromosomes. In recent decades, research has been focused on investigating plants and their phytochemicals as potential antimutagens. The aim of this study was to examine the anti-genotoxic effect of methanolic root and leaf extracts of *Gentiana lutea* against the food mutagens 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) produced in thermally processed meat. To determine the protective potential of extracts, the alkaline comet assay was applied. The results obtained indicated strong anti-genotoxic effect of both extracts against the tested mutagens. The highest inhibition of IQ-induced genotoxicity was recorded for leaf extract (72%). Regarding PhiP, root extract achieved inhibition of 80% of DNA damage, so was more successful than leaf extract. The data obtained in this study stimulates further research of *G. lutea* extracts and its constituents as potential dietary supplements in improving human health.

1. Introduction

Diet can contribute to an increased risk of cancer development, due to the consummation of food mutagens. Food mutagens cause different types of damage in DNA molecule, specifically nucleotide alterations and chromosomal aberrations, by forming carcinogen-DNA adducts. An important class of compounds that are considered a dietary risk factor for development of cancer are heterocyclic aromatic amines (HAAs) [1]. HAAs are formed during the high-temperature cooking of protein rich foods, especially meat and fish, and can significantly increase the risk of different cancers, mainly colon cancer [2]. Numerous studies have been carried out in recent decades in order to identify compounds that might be able to reduce DNA damage. Plants are a rich source of various phytochemicals that can be beneficial for human health, including protection against HAA-induced genotoxicity.

Plants from the genus *Gentiana* are well known for their various biological activities, including antioxidant, antimicrobial, anticancer and radioprotective properties. *Gentiana lutea*, yellow gentian, is widely used in traditional medicine, as well as in the food and pharmaceutical industries. Yellow

gentian root is an officinal drug in many pharmacopoeias for the treatment of mild gastrointestinal diseases [3,4,5].

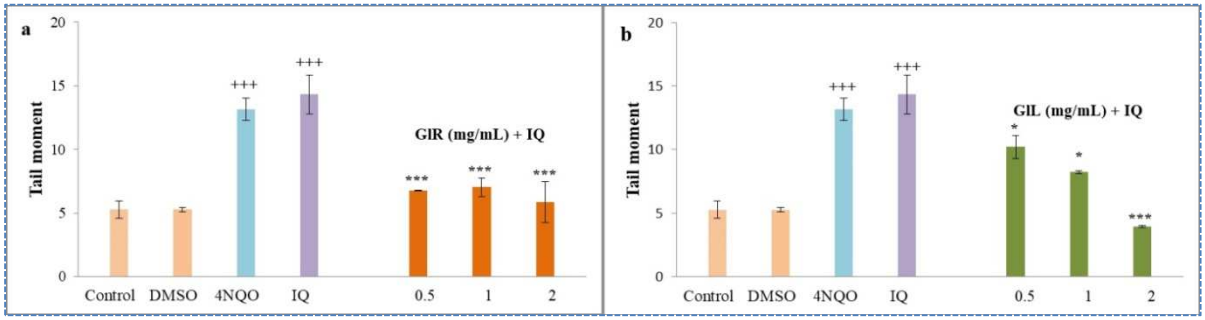
Therefore, the aim of this study was to examine the anti-genotoxic potential of *G. lutea* methanolic root and leaf extracts against 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-1-methyl-6-phenylimiazo[4,5-*b*]pyridine (PhIP) mutagens produced in thermally processed meat.

2. Materials and Methods

Plant material was obtained from the Institute for Medicinal Plants Research Dr Josif Pančić, Belgrade, Serbia. Extract were prepared as previously described by Nastasijević et al. [6]. Chemical characterization of extracts was performed by Ultra Performance Liquid Chromatography (UPLC) as described previously [6]. Results were calculated according to dry weights of root/leaf extract. In order to determine non-cytotoxic concentrations of extracts and food mutagens, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed as previously described by Vasilijević et al. [7]. Genotoxicity and anti-genotoxic potential of extracts and mutagens were tested using the alkaline comet assay as performed by Mitić-Ćulafić et al. [8]. Human hepatocellular carcinoma cells (HepG2) were used as a model system for testing the biological activities. For anti-genotoxicity testing, cells were exposed to co-treatment of extracts and mutagens for 24 h. The statistical analysis was carried out using Mann-Whitney *U* test. Kolmogorov-Smirnov test was used to determine if data were normally distributed. Inhibition of IQ- and PhIP-induced genotoxicity was calculated using the following formula: I (%) = 1- (Nt/Nc)*100, where Nt is the mean value of tail moment of co-treated groups; Nc is the mean value of tail moment of IQ/PhIP.

3. Results and Discussion

Results of UPLC showed the most abundant constituents present in the extracts were gentiopicroside (2.4±0.2%) and loganic acid (0.18±0.01%) in root and leaf extracts, respectively. Preliminary cytotoxicity testing determined the non-cytotoxic doses of extracts and food mutagens: up to 2 mg/mL for extracts; up to 200 µg/mL for mutagens. The highest non-cytotoxic concentrations of extracts and mutagens were tested for genotoxicity to establish the doses of extracts that are non-genotoxic on one hand, and to determine the genotoxic dose of mutagens that induced sufficient DNA-damage, on the other hand. The protective effect of the extracts was tested against IQ (200 µg/mL) and PhIP (100 µg/mL). Results of anti-genotoxicity testing are shown in Figures 1 and 2.



Results are presented as mean values of tail moment ± SD; GlR – *G. lutea* root extract; GlL – *G. lutea* leaf extract; 4-Nitroquinoline N-oxide (4NQO)-positive control (10µM); ***Significant differences between co-treated groups and mutagen; *p<0.05; **p<0.01; ***p<0.001; +++Significant differences in regard to dimethyl sulfoxide (control solution); +p<0.05; ++p<0.01; +++p<0.001

Figure 1. Anti-genotoxic potential of *G. lutea* root (a) and leaf (b) extracts against IQ-induced genotoxicity

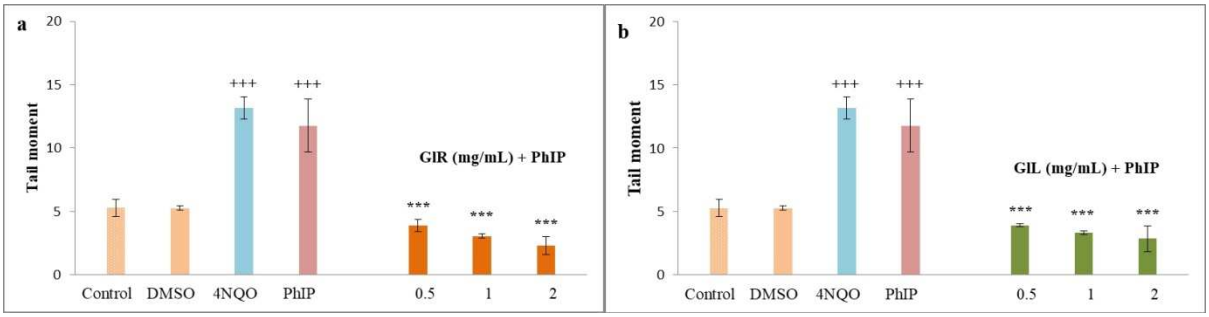


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Figure 2. Anti-genotoxic potential of *G. lutea* root (a) and leaf (b) extracts against PhIP-induced genotoxicity

Exposure of HepG2 cells to IQ and PhIP for 24 h induced significant increase in DNA damage as shown in Figures 1 and 2. The IQ-induced genotoxicity was significantly reduced in the presence of *G. lutea* root extract. Similarly, leaf extract exhibited strong anti-genotoxic potential especially at the highest concentration (2mg/mL) with the inhibition of IQ-induced DNA damage being 72% (Figure 1b, Table 1). Furthermore, both root and leaf extract prevented PhIP-induced DNA strand breaks significantly at all tested concentrations. The highest inhibition of genotoxicity (80%) was recorded for the root extract at the concentration of 2 mg/mL (Table 1)

Table 1. Inhibition of IQ- and PhIP-induced genotoxicity

	IQ			PhIP		
mg/mL	0.5	1	2	0.5	1	2
GlR	53%	51%	58%	67%	73%	80%
GlL	29%	43%	72%	66%	72%	76%

GlR – *G. lutea* root extract; GlL – *G. lutea* leaf extract

Taking into account that HAAs can express mutagenic potential at ng/g levels in cooked foods and can consequently play an important role in the aetiology of human cancer, it is very important to find a way to reduce their harmful effect. Using plants and their phytochemicals as potential protective agents in improvement of human health is a current trend in recent years.

The results obtained in this study are in accordance with available literature data. Viegas et al. [9] showed that flavonoid xathohumol, present in *Humulus lupulus*, exhibited the complete prevention of PhIP-induced DNA damage. Furthermore, significant protective effect of flavonoids quercetin and rutin was demonstrated after DNA damage induced by IQ and PhIP [10]. Rosemary extracts were also effective in the prevention of DNA strand breaks induce by PhIP [11].

A possible explanation for the protective role of *G. lutea* extracts against HAAs may lie in the fact that yellow gentian is known for its antioxidant properties. In previous studies, root extracts expressed quite strong antioxidant activity, with the IC_{50} value at 20.6 µg/mL recorded in the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay [6, 12]. Considering the fact that aside from forming the DNA-adducts, HAAs might also generate reactive oxygen species that cause oxidative DNA damage [13,14], using compounds with strong antioxidant potential is a promising way to protect the integrity of genome.

4. Conclusions

The results obtained in this study point to methanolic root and leaf extracts of *G. lutea* as potential protective agents against the thermally produced food mutagens, IQ and PhIP. Both extracts expressed statistically significant anti-genotoxic effects at all tested concentrations. The highest inhibition of IQ- and PhIP-induced genotoxicity was recorded for leaf extract (72%) and root extract (80%), respectively. The data obtained are promising, indicating yellow gentian is suitable as a potential dietary supplement to improve human health. In accordance with our results, further research should be focused on investigating the protective effect of *G. lutea* extracts and constituents in *in vitro* and *in vivo* model systems.

Acknowledgement

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project no.172058).

References

[1] Goldam R and Shields P G 2003 *J. Nutr.* **133** 965S−973S
[2] Sugimura T, Wakabayashi K, Nakagama H and Nagao 2004 M *Cancer Sci.* **95** 290−9
[3] Pan Y, Zhao Y-L, Zhang J, Li W-J and Wang Y-Z 2016 *Chem. Biodivers.* **13** 107−50
[4] Mirzaee F, Hosseini A, Jouybari H B, Davoodi A and Azadbakht M 2016 *J. Tradit. Complement. Med*. **7** 400−8
[5] Menković N, Juranić Z, Stanojković T, Raonić-Stevanović T, Šavikin K, Zdunić G and Borojević N 2010 *Phytother. Res*. **24** 1693−6
[6] Nastasijević B, Lazarević-Pašti T, Dimitrijević-Branković S, Pašti I, Vujačić A, Joksić G and Vasić V 2012 *J. Pharm. Biomed. Anal.* **66** 191−6
[7] Vasilijević B, Knežević-Vukčević J, Mitić-Ćulafić D, Orčić D, Franciškovic M, Srdić-Rajić T, Jovanović M and Nikolić B 2018 *Food Chem. Toxicol.* **112** 118−25
[8] Mitić-Čulafić D, Žegura B, Nikolić B, Vuković-Gačić B, Knežević-Vukčević J and Filipič M 2009 *Food Chem. Toxicol.* **47** 260−6
[9] Viegas O, Žegura B, Pezdric M, Novak M, Ferreira I M P L V, Pinho O and Filipič M 2012 *Food Chem. Toxicol.* **50** 949−55
[10] Kurzawa-Zegota M, Najafzadeh M, Baumgartner A and Anderson D 2012 *Food Chem. Toxicol.* **50** 124−9
[11] Žegura B, Dobnik D, Hojnik Niderl M and Filipič M 2011 *Environ. Toxicolo. Pharmacol.* **32** 296−305
[12] Azman N A M, Segovia F, Martìnez-Farré X, Gil E and Almajano M P 2014 *Antioxidants* **3** 455 −71
[13] Murata M, Kobayashi M and Kawanishi S 1999 *Jpn. J. Cancer Res.* **90** 268−75
[14] Maeda H, Sawa T, Yubisui T and Akaike T 1999 *Cancer Lett*. **143** 117−21